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ANGIOGENESIS IN HAND CHONDROMA: AN IMMUNOHISTOCHEMICAL STUDY

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Hand chondroma is a particular cartilagineous tumour, being clinically benign, but morphologically malignant. This study investigates the expression of VEGF together with other growth factors and proliferation markers such as TGFβ2, Ki-67, TNF, FGF1, P53 in 8 cases of hand chondroma treated with courettage, in order to define the ethiopathogenesis of this tumour and the clinical significance of the resulting immunohistochemical profile, with particular respect to angiogenesis. VEGF was expressed in all cases; 5 cases were positive for TFGβ2 and 3 for PDGF. None of the other factors was expressed. On the basis of histologic results a specific model of tumour progression based on the indicators of angiogenesis could be related to hand tumours, in which VEGF expression should be the first stadium of the tumour aggressiveness, and the following PDGF, TGF 2 expression should be accompanied with a morphological outline worsening. Nevertheless the non constant expression of these indicators and the absent expression of proliferated indicators can explain the scant tendency to the relapse in presence of accurate curettage. It is important to remember that the cellular polymorphism typical of the cartilaginous tumours does not allow the application of an only oncogenesis model.

Chondroma, the second commonest benign cartilaginous tumour after osteochondroma, is composed of normal-appearing hyaline cartilage. In contrast with chondrosarcoma, which occurs in the axial skeleton, chondroma has a specific predisposition to the appendicular skeleton and it is the most common benign tumour of the hand. This lesion has a limited potential for malignant transformation and there is still a real difficulty in differentiating chondromas from low-grade chondrosarcomas. (1) Chondromas of the hand represent a group of lesions which was studied with different immunohistochemistry (IIC) to evaluate the immunophenotypical behaviour in comparison with our IIC previous study of chondrosarcoma (2) In fact, the growth and progression of a tumour proceed through the production of different growth factors which can also be used as biologic markers of the tumour identification. VEGF is an homodimeral heparin-binding glycoprotein correlated to the tumoral angiogenesis. In fact a tumour creates an internal equilibrium between the production of both angiogenetic and endogenous antiangiogenetic factors. The overexpression of angiogenetic factors, such as Vascular Endothelial Growth Factor (VEGF), is an adverse prognostic marker in carcinomas, for its involvement in the progression of metastasis. (3)

The unbalance toward an angiogenetic factor predominance represents the first step in the progression of malignant tumours. A radial growth over 3 mms requires, in fact, a new vascularization. VEGF stimulates the proliferation of the endothelial cells, but it is also involved in the increase of the vascular permeability. The majority of tumours of both epithelial and mesenchymal origin show a VEGF-related neovascularisation proportional to both tumour growth and metastatic behaviour. Few data about the potential role of angiogenesis in non-vascular neoplasm are reported in literature. A paper of our group has recently demonstrated the direct correlation between expression of VEGF and malignant course of cartilaginous neoplasms. Hand chondroma represents a specific tumour for the well known discrepancies existing between its morphologic picture and its clinical behaviour (4-8). The aim of the present study is to investigate the expression of VEGF together with other growth factors and proliferation markers such as TGFβ2, Ki-67, TNF, FGF1, P53 in hand chondroma in order to define the ethiopathogenesis of this tumour and the clinical significance of the resulting

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immunohistochemical profile.

MATERIALS & METHODS

Eight cases of hand chondroma underwent surgery at the Department of Orthopaedics and Traumatology of the Catholic University of Rome between 1997 and 2009. Age, sex, tumour location, symptoms declared by patient upon admission, treatment, treatment complications, local relapses and survival were all taken into consideration. Clinical charts were reviewed to evaluate age, sex, specific location, symptom at presentation, treatment, complication of treatment, survival. All patients had a preoperative radiographs which were accurately studied. Slides of histological sections were obtained from paraffin-embedded blocks retrieved from the archives of the Department of Anatomic Pathology and Histology of our university, stained with hematoxylin and eosin and observed by the same pathologist (GF, EDR).The histologic diagnosis was made in all cases after reviewing the clinical and radiologic data. The expression of antibodies against growth factors (VEGF, TNF, ...) and proliferation markers (Ki-67...) were assessed using the following immunohistochemical (IHC) technique. All specimens were fixed in 10% buffered formaldehyde, embedded in paraffin and the 5 μm-thick sections were stained with hematoxylin-eosin. IHC staining was performed on sections taken from the paraffin-embedded tissue. 5-μm sections were deparaffinised in xylene then rehydrated. Endogenous peroxidise activity was blocked in 1% normal H2O2 methanol.

After a short rinse in phosphate-buffer solution (PBS), the sections were preincubated with1% normal horse seum Seven markers were evaluated: VEGF(Dako, Denmark), Ki-67(Dako,Italy), TGFB(Dako, Denmark), TGF1(Ylem, Italy), TNF,(Ylem, Italy), PDGF (Dako, Denmark), P53 (Dako, Denmark), according to the manufacturers instructions. Monoclonal antibodies were applied in a 1:50 or 1: 100 for Ki-67 for 1 hour. They were treated with the avidin-biotin peroxidase complex (ABC-Elite Kit; Vector Laboratories, Burlingame, CA). The reaction products in the sections were visualized with freshly prepared 0.01% 3-3-diaminobenzidine tetrahydrochloride (DAB; Sigma) containing 0.02% H2O2. The sections were counterstained with Harris hematoxylin.

All cases were reviewed by a single pathologist (GF) and those cases where a univocal diagnosis was not possible, were discussed by the other pathologists (EDR, LL, GFZ) until a final agreement was achieved. The diagnosis for each case was carried out by integrating the clinical radiographic and histological data. The growth factors and cellular proliferation markers were analysed through immunohistochemistry. Only when at least 20% of the cells showed a convincing positivity, the reaction for the selected antibodies was defined as positive.

Statistical analysis

Sensitivity (SE), specificity (SP), diagnostic accuracy (DA), were calculated .The statistical analysis was performed by using a commercially available statistic software package (SPSS 10.0 for Windows ® - SPSS Inc., Chicago, IL, USA). The chi-square test was used for categorical variables and a p value lower than 0.05 was considered as significant. The Fisher exact test was used to assess the differences in marker expression among the group.

RESULTS

All patients were followed-up for a period ranging from 7 to 165 months (mean 74.7). Clinical and IHC data are reported in Table 1 and Table 2 respectively. Median age at diagnosis was 37.1 (range 10-49). Six patients were females and 2 males. Pain was always present at the involved site; local swelling was present in 3 cases and a pathologic fracture occurred as first symptom in 2 cases (25%).

Curettage of the bony lesion as surgical treatment was performed with synthetic bone graft in 6 out of 8 (75%). In 2 cases (25%), the residual hole was not filled. In one case a relapse of the disease occurred after the curettage. The follow-up of these patients resulted disease-free until now. The histological diagnosis were in all 8 cases consistent with enchondroma. In all cases excision of the tumour was macroscopically achieved. The expression of the immunohistochemical factors analyzed in the group, are summarized in Table 2. VEGF was expressed in all cases; 5 cases were positive for TFGβ2 and 3 for PDGF. None of the other factors was expressed.

DISCUSSION AND CONCLUSIONS

Enchondroma represents the most common primary benign skeleton neoplasm. The hands, and predominantly the phalanges, are a common site of occurrence of this tumor unlike its malignant counterpart which is uncommon in this body site.(9-10) As reported in literature, the possibility of a malignant transformation of a hand chondroma is extremely rare (11-12) A differential diagnosis between malignant and benign tumours is sometimes very difficult also at the microscopic level.

It is known that enchondromas of hands and feet display greater cellularity and nuclear atypia than is sustained elsewhere. Because of the contrast between the atypical histologic appearance of these tumours and their favourable biologic behaviour, we have decided to focus on this group and evaluated some immunohistochemical stains in comparison with chondroma and chondrosarcoma of our previous papers. In our series of hand chondroma, all eight cases resulted positive for VEGF and 5 were positive for TGFB2. These data were closer to the IHC for chondrosarcoma rather than the data of chondromas. The lack of expression of Ki-67 and P53 may be regarded as an important clue of the good tumour biologic behaviour (13-16).

The p53 is an oncosoppressor gene and one of its mutation is correlated to the tumour growth promotion. The Ki-67 is used for evaluating the proliferate fraction in the neoplastic lesions, being expressed in all cellular cycle phases unless in the G0 one. In the previous study,

Table I. *Number of cases with expression of the growth factors in all examined groups.*

Patients	Sex	Age	Symptoms	Location	Relapse	Treatment
Pt 1	F	34 years	Fracture	IIth phalanx	Yes	Courettage+bone filling
Pt 2	F	10 years	Fracture	IIth phalanx	No	Courettage+bone filling
Pt 3	F	37 years	Pain	IIIth phalanx	No	Courettage
Pt 4	F	49 years	Pain	IIIth phalanx	No	Courettage+bone filling
Pt 5	F	45 years	Swelling	IIth phalanx	No	Courettage
Pt 6	F	36 years	Pain	IIIth phalanx	No	Courettage+bone filling
Pt 7	M	39 years	Swelling	IIth phalanx	No	Courettage+bone filling
Pt 8	M	47 years	Swelling	IIIth phalanx	No	Courettage+bone filling

Table II. *Correlation of angiogenetic factors between enchondroma, chondroma of the hand and chondrosarcoma.*

Histology	cases	VEGF	KI-67	TGFß2	TNFa	PDGF	FGF1	P53
Chondroma	8	1	0	1	0	0	0	0
Hand Chondroma	8	8	0	5	0	3	0	0
Chondrosarcoma	13	9	4	4		4	1	3

For chondrosarcoma has been shown that radiological features displayed on conventional X-ray do not improve the ability to differentiate between enchondroma and grade 1 chondrosarcoma . So, for these two entities, both of them cartilagine-forming bone neoplasms with a characteristic lobular pattern is essential a final histological diagnosis in terms of treatment and prognosis.

according to the literature (13), the over expression of Ki63 e p53 was related to unfavourable course (developing of local relapse and pulmonary metastasis). In the present study these markers were negative in all cases, even in the case that developed local relapse, indicating that the favourable clinical course of hand chondroma is inversely related to these indicators. So, KI-67 expression, associated with a proliferate phase of the cells, was positive in 4/13 cases of chondrosarcoma but negative in all hand chondroma and P-53 suggesting a role in the progression of cartilaginous tumours was positive in 3/13 chondrosarcomas and in none hand-chondroma.

The cartilaginous tumours, likewise to the articular cartilage, are practically a non vascularised tissue. The correlation between VEGF expression and tumour grading in the different histotype (chondroma, low grade and high grade chondrosarcoma) of cartilagineous tumours was largely examined in our previous paper, showing a certain accuracy in the distinction among the low and high degree lesions.

The adult cartilage is generally considered a tissue without vascolarization, although the hypertrophic layer produces activators of angiogenesis. McGough et al. have reported also the bFGF expression as an important factor pro-angiogenetico with a positive coloration both in the benign lesions and in the malignant ones. Based on VEGF expression, combined with the expression of other factors creating a small immunopanel, aFGF, p53, Ki-67, PDGF, TGF were proposed as a model of tumour progression with the indicators of angiogenesis and

cellular proliferation expression (17-18).

Nevertheless the non constant expression of these indicators and the absent expression of proliferated markers, can explain the scant tendency to the relapse in presence of accurate curettage. It is important to remember that the cellular polymorphism typical of the cartilaginous tumours does not allow the application of an unique oncogenesis model. As discussed in multiple papers, VEGF inducing endothelial cell proliferation and increasing permeability and extravasation, is correlated with growth, progression and metastatic potential, with a lower survival rate in patients with VEGF positive.

The VEGF behaviour in the hand lesions is different and immunohistochemically ambiguous: they are morphologically malignant tumours but with a clinical behaviour of absolute and complete benignity. Taking into account the VEGF behaviour in hand tumours we think that the model can not be applied in automatic way to the hand tumours, because, we would have to suppose that the VEGF expression, always present in these forms, should be the first stadium of the tumour aggressiveness, in which the following PDGF, TGF 2 expression should be accompanied with a morphological outline worsening.

The expression of TGF β2 and the PDGF was also evaluated. The TGF β2 is a powerful mitogen agent positive in more than 50% of analyzed cases. Our data suggest that the it can be considered as a progression model of the cancer as releved in malignant colon cancer. The VEGF expression of the neoplastic cells can be considered as the first stadium with which the tumour starts to show the high grade characteristics of malignancy. The PDGF, TGF 2, Ki-67 and p53 expression represents a belated event in the oncogenesis, when the tumour assumes a more aggressive behaviour. This proposed model of tumour progression based on the indicators of angiogenesis and cellular proliferation expression, is not applied in automatic way to the hand tumours, because if it so was, we would have to suppose that the VEGF expression, always shown in these forms, should be the first stadium of the tumour aggressiveness, in which the following PDGF, TGF 2 expression should be accompanied with a morphological outline worsening. Nevertheless the non constant expression of these indicators and the absent expression of proliferated indicators can explain the scant tendency to the relapse in presence of accurate curettage. It is important to remember that the cellular polymorphism typical of the cartilaginous tumours does not allow the application of an only oncogenesis model.

In conclusion, the VEGF expression correlates with the presence of cellular polymorphisms that are an expression of malignancy in the central chondrosarcomas, while in the hand chondroma these polymorphisms don't correlate with the clinical malignancy.

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